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Maternal Immunity and Antibody Response of Neonatal Mice to Pneumococcal Type 19F Polysaccharide

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The effect of immunization of mothers on the antibody response of their young to pneumococcal type 19F polysaccharide was studied. When 2-week-old BALB/c mice from mothers immunized with 23-valent pneumococcal vaccine during gestation were given an additional dose of the same vaccine, mouse pneumococcal antiserum, or both, they produced higher titers of antibodies to the 19F polysaccharide (1.87 to 4.66 μg of 19F immunoglobulin M [IgM] antibody per ml of serum; 0.45 to 0.81 μg of IgG antibody per ml of serum) than the control group that did not receive any treatment after birth (0.69 μg of 19F IgM antibody per ml; 0.28 μg of 19F IgG antibody per ml) ($P < 0.01$). Furthermore, all 11- to 12-week-old monkeys that received an additional dose of 23-valent vaccine, pneumococcal immunoglobulin, or both produced statistically higher titers of IgG antibody to the 19F polysaccharide than did controls at various ages. The titers (micrograms of IgG antibody per milliliter of serum) were as follows: vaccine group, 7.12 ± 0.96; control group at 4 months of age, 3.82 ± 0.74 ($P < 0.01$); immunoglobulin-treated group, 6.85 ± 0.76; vaccinated and immunoglobulin-treated group, 7.80 ± 1.40; control group at 3 months of age, 3.01 ± 0.61 ($P < 0.01$). These results suggest that immunization of mothers under certain conditions, such as with an optimum dose of antigen at a critical period of gestation or postnatal development, could provide young infants with an enhanced antibody response to pneumococcal polysaccharide immunogens.

One of the most common *Streptococcus pneumoniae* types causing pneumococcal disease in young children is type 19F, which ranks 10th in frequency among all pneumococcal isolates (5, 20, 25, 28, 33). Types 6, 14, 19F, and 23F account for 50 to 60% of pneumococcal isolates from infants and children (2), whereas type 19F is the most prevalent type isolated from the middle ear fluid of children with acute otitis media (423 [23%] of 1,837 strains) (14). Multiple antibiotic resistance has been reported in serotypes 19F, 19A, 3, 6, 14, and 23F (15, 16, 18, 26). Type 19F polysaccharide (PS) is contained in the 23-valent pneumococcal vaccine. However, it has induced a low antibody response in young children. It is less stable than other types of pneumococcal PS in the vaccine (21). These factors, including the high prevalence of pneumococcal 19F diseases, emergence of strains that are resistant to various antibiotics, and low immune response of pediatric pneumococcal types (e.g., types 6 and 19F), have emphasized that further studies are required to increase the immune response of young children to pneumococcal 19F and other types of PS.

Immunization of mothers with type 19F PS during gestation and/or lactation has induced a higher antibody response in the offspring. Injection of female mice with type 19F PS monovalent immunogen before their mating also induced a higher antibody response in young mice (19, 23). Furthermore, combined passive immunization with immunoglobulin and active immunization of infant mice with monovalent PS may elicit sufficient 19F antibody formation for protection against infection during early life (23). Maternal serum antibodies, including immunoglobulin M (IgM), IgG, and IgA, have been reported to be present in colostrum; these immunoglobulins, which are transferred to nursing neonates, are still largely intact (10). Immunization with bacterial PS or PS-protein conjugate vaccines during early life induced humoral antibodies and provided effective protection against infection (30, 32, 34). These studies have suggested the possible application of maternal immunization with pneumococcal vaccine to enhance the antibody response of young infants to 19F PS and other PS antigens for protection of pneumococcal infection during childhood.

We report here our study on the effect of immunization of mothers with a 23-valent pneumococcal vaccine during gestation on the subsequent antibody response of young infants to pneumococcal 19F PS. Influences on maternal immunity were investigated further in a monkey model.

MATERIALS AND METHODS

Materials. Pneumococcal type 19F PS and the 23-valent vaccine were obtained from Merck Sharp and Dohme, West Point, Pa. The chemical characteristics of type 19F PS have been reported previously (17). The 23-valent vaccine consists of 25 μg each of PS types 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F, and 33F in 0.5 ml of saline. Mouse and monkey IgG and mouse IgM were obtained from Miles Biochemicals, Inc., Elkhard, Ind. Cyanuric chloride was obtained from Aldrich Chemical Co., Milwaukee, Wis. Brij, alkaline phosphatase-rabbit anti-mouse IgG and rabbit anti-mouse IgM conjugates, *p*-nitrophenyl phosphate, and poly-L-lysine were purchased from Sigma Chemical Co., St. Louis, Mo. The peroxidase-conjugated IgG fraction of goat anti-monkey IgG was obtained from Cappel Laboratories, West Chester, Pa. Male and female BALB/c mice, 8 to 10 weeks of age, were obtained from the Small Animal Production Section of the National Institutes of Health, Bethesda, Md. Pneumococcal cell wall PS was kindly supplied by T. Y. Liu and L. Y. Wang, Center for Biologics, Bethesda, Md. Human hyperimmune globulin to pneumococcal types 1, 3, 6, 7, 8, 9, 12, 14, 18, 19, and 23 was obtained from George R. Siber, Massachusetts Public Health Biologic Laboratories, Boston.

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The globulin contained 626 ng of type 19F antibody nitrogen per ml (38). Meningococcal group A PS was obtained from Connaught Laboratories, Inc., Swiftwater, Pa. Rabbit meningococcal group A antiserum was obtained from the Center for Biologics Research and Evaluation, Bethesda, Md. It was prepared by repeated intravenous injection of rabbits with Formalin-treated whole meningococcal group A (strain A1 suspension [7]) and had a serum titer of greater than 1:64 as determined by immunodiffusion (29).

Mouse pneumococcal antiserum was prepared by injection of 8- to 10-week-old mice with 5 μg of 19F PS-R61 membrane protein conjugate, once per week for 3 weeks. The 19F antibody concentrations were approximately 40 μg of IgM antibody and 18 μg of IgG antibody per ml of serum, as measured with an enzyme-linked immunosorbent assay (ELISA).

Type 19F PS was covalently linked to a carrier protein by the modified carbodiimide coupling method (24, 36). In brief, 19F PS was activated with cyanogen bromide at pH 10.5 for 6 min and reacted with 0.1 M 6-aminocaproic acid. The PS–6-aminocaproic acid complex and the membrane protein (50 mg per 10 ml in 0.1 M sodium acetate buffer [pH 5.0]) were mixed, and coupling was carried out with 1-ethyl-3(3-dimethyl-amino propyl)-carbodiimide HCl (250 mg/ml) for 1 h at 4°C. The reaction mixture was passed through a Sepharose 4B column, and the void volume peak was retained. The concentrations of protein and PS, their ratios, and the molecular size profiles of the PS-protein conjugates were determined as described previously (22).

Immunological methods. Antibody levels in serum were determined by a modified ELISA (9, 19, 37). In brief, 0.1 ml of PS antigen (1 mg/ml) was added to 0.5 ml of 0.01 M sodium hydroxide solution. Next, 0.5 mg of cyanuric chloride and 0.1 ml of 0.1% poly-L-lysine were added. The solution was mixed thoroughly and kept at 4°C for 2 h and then adjusted to 1 μg of PS/ml in phosphate-buffered saline (PBS) (pH 7.4). Polystyrene microdilution plates were filled with 0.1 ml of PS-polylysine complex per well, kept at 4°C for 17 h, and washed with detergent solution (0.05% Brij) three times. The test antiserum samples were preabsorbed to remove cell wall PS antibody by adding 1 μg of cell wall PS to 1 ml of a 1:50 dilution of serum and incubated at 25°C for 60 min. Mouse and monkey antiserum standards and/or antiserum samples diluted 1/100 to 1/300 in PBS containing 5% fetal calf serum and 0.01% sodium azide were added to plates and mixed with shaking at 25°C for 2 h. Alkaline phosphatase conjugated to rabbit anti-mouse IgG or IgM and diluted 1/1,000 in PBS was added to each well. The microdilution plates were returned to the shaker for 2 h. After the plates were washed, 1-mg/ml *p*-nitrophenyl phosphate in 1 M Tris buffer (pH 9.8) containing 0.3 mM magnesium chloride was added to each well. After 30 or 60 min, the reaction was stopped by the addition of 0.05 ml of 4 N sodium hydroxide. The optical density was measured spectrophotometrically at 405 nm. The ELISA for measuring monkey IgG antibody was performed under similar conditions with peroxidase conjugated with goat anti-monkey IgG antibody and *o*-phenylenediamine as the substrate.

The effects of coating the microdilution plates with PS-polylysine complex, sample antibody concentration, duration of incubation, and temperature were studied to determine the optimum conditions for the ELISA. The PS antigen-antibody reaction by the alkaline phosphatase-conjugated mouse IgM or IgG activity was optimum when microdilution plates were coated with 1 μg of PS per ml and kept at 4°C for 17 h. The standard curves of mouse IgM and IgG antibodies were proportional and linear at concentrations between 0.05 and 0.4 μg/ml. A proportional and linear relationship on the standard curve of monkey IgG was observed at concentrations between 0.1 and 1.0 μg/ml.

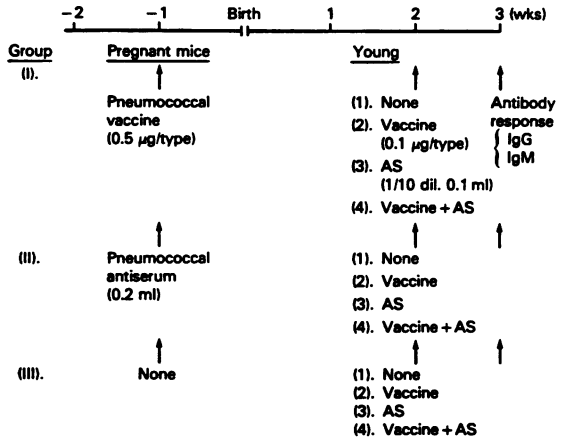

FIG. 1. Experimental design for evaluation of maternal immunization on the antibody response of young mice to pneumococcal type 19F PS. Fourteen days after birth, the young from mothers that were immunized with pneumococcal vaccine (group I), mouse pneumococcal antiserum (AS) (group II), or saline (group III) received 0.1-ml i.p. injections of saline (subgroup 1), vaccine (0.1 μg of each PS) (subgroup 2), a 1/10 dilution of mouse pneumococcal antiserum (subgroup 3), or both the vaccine and antiserum (subgroup 4). The experimental conditions are described in detail in Materials and Methods.

Immunization of mothers and passive immunity. (i) Mouse study. Figure 1 shows the experimental design for the evaluation of the effect of immunization of mothers and passive immunity on the antibody response of young infants. Pregnant mice at 2 weeks of gestation were given the following subcutaneous injections: the first group received a 23-valent pneumococcal vaccine (0.5 μg of each PS in 0.2 ml), the second group received 0.2 ml of mouse pneumococcal antiserum, and the third group received the same amount of saline. Fourteen days after birth, young mice were given one of the following treatments intraperitoneally (i.p.): (i) saline (0.1 ml), (ii) pneumococcal vaccine (0.1 μg of each PS in 0.1 ml), (iii) mouse pneumococcal antiserum (0.1 ml of a 1/10 dilution), or (iv) both vaccine and antiserum (ii and iii) given at different sites. In another experiment, groups of eight pregnant mice received the vaccine (0.5 μg of each PS in 0.2 ml) at 14 days of gestation. Fourteen days after birth, young mice were given one of the following treatments i.p.: (i) saline (0.1 ml), (ii) meningococcal group A PS (0.1 μg per 0.1 ml), (iii) rabbit meningococcal group A antiserum (1/10 dilution, 0.1 ml), or (iv) normal mouse antiserum (0.1 ml of a 1/10 dilution). Seven days later, serum samples from all young mice were obtained, and the antibody levels were determined by using the ELISA.

(ii) Monkey study. Some experiments conducted in the mouse study were further examined by using monkeys (Fig. 2). Altogether, 40 pregnant rhesus monkeys, 12 to 15 months of age and 2 to 3 weeks before delivery, were injected subcutaneously with pneumococcal vaccine (25 μg of each PS in 0.5 ml). After birth, young monkeys at 11 to 12 weeks of age from vaccinated mothers were given one of the following treatments i.p.: (i) saline (0.5 ml), (ii) an additional dose of pneumococcal vaccine (5 μg of each PS in 0.5 ml) at

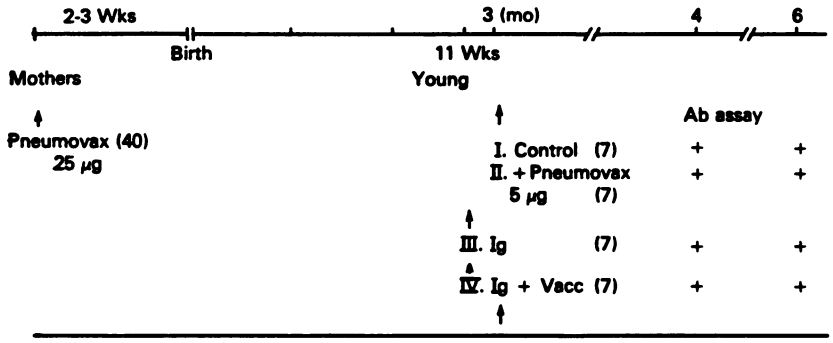

FIG. 2. Experimental design for evaluation of the antibody responses of young monkeys to pneumococcal type 19F PS. After birth, 11- to 12-week-old monkeys (seven monkeys per group) from vaccinated mothers were injected i.p. with 0.5 ml of saline (group I), pneumococcal vaccine (5 μg of each PS) at 3 months of age (group II), pneumococcal immunoglobulin (Ig) (1 ml of each) at 11 weeks of age (group III), or the pneumococcal immunoglobulin at 11 weeks of age plus the vaccine at 12 weeks of age (group IV).

12 weeks of age, (iii) pneumococcal immunoglobulins (1 ml of each) at 11 weeks of age, or (iv) pneumococcal immunoglobulins (1 ml of each) at 11 weeks and vaccine (5 μg of each PS in 0.5 ml) at 12 weeks of age. The IgG antibody response to type 19F PS was measured in maternal serum samples before vaccination, at delivery, and 4 and 6 months after delivery, and serum samples from the young at birth and at 11 weeks and 3, 4, and 6 months of age.

RESULTS

We evaluated the effects of immunization of mothers and passively acquired neonatal immunity on the IgM and IgG isotype responses of mice and monkeys to the pneumococcal type 19F PS.

Studies in mice. The effects of immunization of mothers and passive immunity on the antibody response of young mice to type 19F PS are shown in Fig. 3. When young mice from vaccinated mothers received an additional dose of pneumococcal vaccine, antiserum, or both vaccine and antiserum, they produced significantly higher levels of IgM and IgG antibodies than did the control group that received no treatment after birth. The young mice that received an additional dose of vaccine produced IgM and IgG antibody titers that were 6.8 and 2.9 times higher, respectively, than those of the control group. Treatment of young mice with meningococcal group A PS, rabbit meningococcal group A antiserum, or normal mouse antiserum did not affect their antibody responses to the 19F PS.

Injection of pregnant mice with pneumococcal antiserum neither stimulated nor suppressed the antibody response of the young that received an additional dose of antiserum or vaccine plus antiserum after birth. Thus, passive immunization with pneumococcal antiserum or combined passive and active immunization did not induce observable harmful immunologic effects. When the vaccine, pneumococcal antiserum, or both were injected directly into young mice, a low 19F antibody response was seen.

Studies in monkeys. The IgG antibody responses of monkey mothers to the 19F PS is shown in Fig. 4A. Preimmune serum samples showed a 19F antibody level of 1.23 to 1.85 μg/ml. At delivery, the 19F antibody increased to 5 to 5.9 μg/ml, a 2.7- to 4.6-fold increase in antibody titer. The 19F antibody levels in serum remained at elevated levels for several months and then gradually decreased. Six months after vaccination, the IgG antibody titers were 1.4 to 2.7 times the preimmunization levels.

The IgG antibody responses of young monkeys to 19F PS are shown in Fig. 4B. At birth, the infants had 19F IgG antibody levels of 3 to 4 μg/ml, slightly lower than the antibody level of the mothers. The 19F antibody levels in the control group gradually decreased over 6 months. Young monkeys that received an additional dose of vaccine had significantly higher 19F antibody titers at 4 months of age than did the control group. Those that received pneumococcal immunoglobulin at 11 weeks of age had significantly elevated antibody titers at 12 weeks; the titers gradually decreased over 6 months. The group that received immunoglobulin plus the vaccine showed the highest 19F antibody response at 4 months of age. The antibody titer was 2.3-fold higher than that of the control group. At 3, 4, and 6 months of age, the antibody titers from the group that received immunoglobulin plus the vaccine were higher than that of the control group.

DISCUSSION

Most bacterial PSs do not induce significant antibody formation when given to infants. A possible means whereby infants could be protected from these infections in early life is through immunization of the mothers during gestation, with the development of high IgG antibody levels in serum that can be transferred to the infant through the placenta and the mother's milk. For example, infants born of mothers with high levels of specific antibodies to group B streptococcal type III PS are less susceptible to type III infection than are other infants (3). Passive immunization was reported to be effective for treatment of pneumococcal, meningococcal, and *Haemophilus influenzae* type b infections (38). Furthermore, passive immunization combined with active immunization induced high antibody responses in neonates (11, 40) and may be applicable to the prevention of bacterial infections.

Recently, many studies have supported a concept that the neonatal immune system is susceptible to regulation by anti-idiotypic antibodies (31, 35). Priming effects of maternal anti-idiotypes have been transmitted in milk (39), and in utero sensitization to transferred antibodies has been induced (27). These studies have indicated that anti-idiotypic antibodies or idiotypes (primary antibodies) can cross the placenta and alter the immune response of offspring. Maternal antibodies and anti-idiotypic antibodies can prime the infants for protection against a bacterial infection; e.g., infants whose mothers were immunized with tetanus toxoid

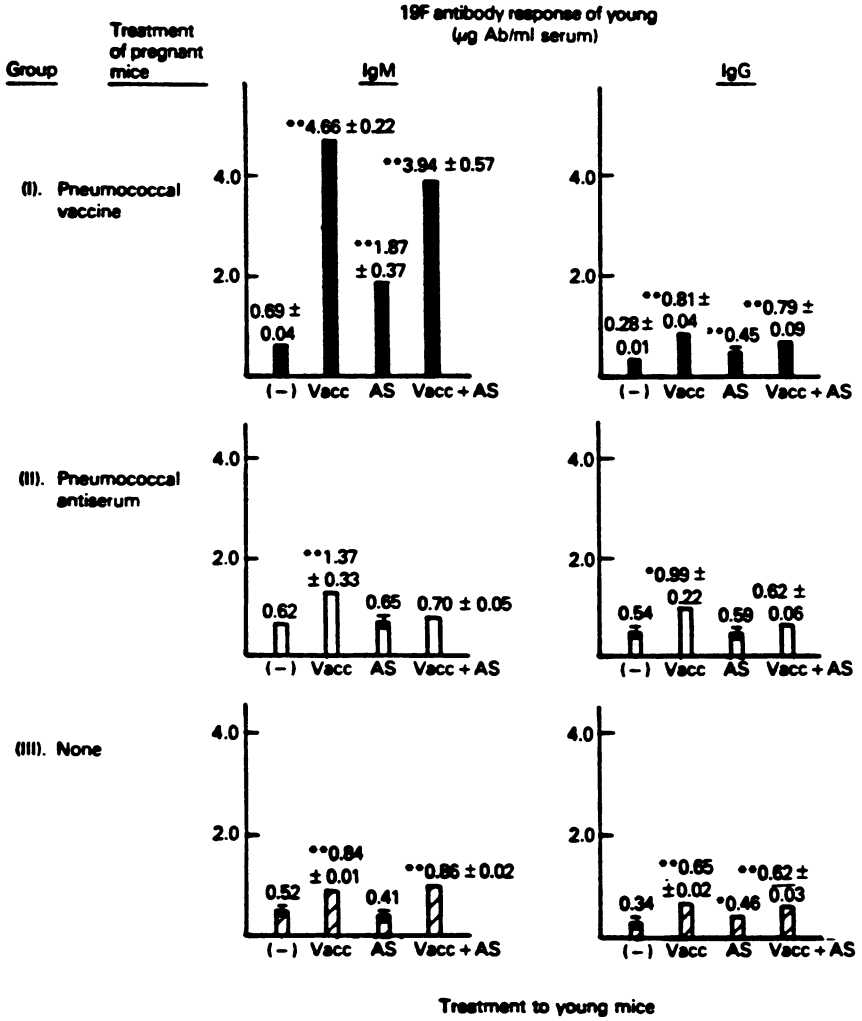

FIG. 3. Effects of immunization of mothers and passive immunity on 19F antibody response. Mean antibody levels ± standard errors between subgroups were compared by using a two-tailed Student *t* test. The antibody responses of young from pregnant mice immunized with pneumococcal vaccine (group I), antiserum (group II), or saline (group III) that received an additional dose of vaccine (Vacc), antiserum (AS) or vaccine plus antiserum were compared with those of young mice receiving no treatment (−). *, $P < 0.05$; **, $P < 0.01$. Each group consisted of six to eight animals.

during pregnancy responded better to subsequent immunization than did infants of nonimmunized mothers (8). The administration of idiotypic antibody to *Escherichia coli* K13 PS to infants may serve a dual function in providing passive protection as well as priming for protective antibodies upon subsequent antigen exposure (39). Maternal antibody may not always have a priming effect; e.g., when high cord blood antibody levels existed and immunization of pertussis toxin antigen was given to the infants, reduced IgG antibody responses were observed (6). The results in the present study are similar to those in studies of *E. coli* K13 PS or tetanus toxoid antigen in that the maternal antibody primed the immune response of young, possibly via regulation by an idiotypic network.

In the present study, when young mice from mothers immunized with pneumococcal vaccine during gestation received an additional dose of vaccine, antiserum, or both, they produced significantly higher levels of IgM and IgG antibodies to type 19F PS than did the control group that did not receive any treatment after birth. Treatment of young mice with an unrelated bacterial PS (e.g., meningococcal group A PS), an unrelated antiserum (e.g., meningococcal group A antiserum), or normal mouse antiserum had no effect on their antibody response to 19F PS. Thus, the immunization of the mothers resulted in priming young infants to enhance their antibody response to the vaccine.

It has been reported that postnatal infusion of human IgG containing high titers of antibodies to group B streptococcal PSs prevented bacteremia and improved the survival of group B streptococcus-infected infant rhesus monkeys (12). Furthermore, immunization of pregnant women with PS vaccine from a group B streptococcus can increase the level of maternal IgG antibody that will cross the placenta and passively protect the neonate (1, 4, 13). Thus, passive immunization with immunoglobulin to PS antigen is efficient in preventing perinatal infections in infants. The results of the present study further support the notion that young monkeys from immunized mothers, who received a dose of vaccine, pneumococcal immunoglobulin, or both, induced higher type 19F IgG antibodies than did the control group. These studies suggest that immunization of mothers will prime the young to respond effectively to PS antigens. The results further suggest that immunization of mothers under certain conditions, such as with an optimum dose (although

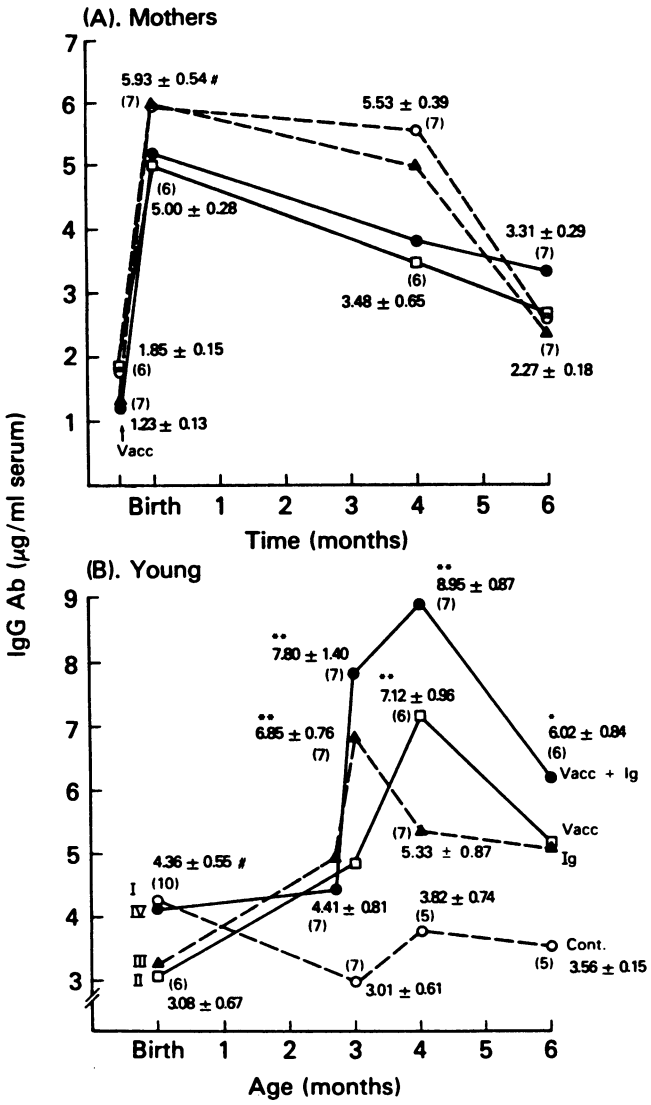

FIG. 4. IgG antibody response of monkeys to type 19F PS. Results are shown as mean antibody (Ab) levels ± standard errors in mothers (A) and young (B). The antibody responses of young from immunized mothers receiving additional treatment after birth (group II, III, or IV; Fig. 1) were compared with that of young receiving no treatment (group I, control). *, $P < 0.05$; **, $P < 0.01$. The numbers of animals are shown within parentheses. Vacc, vaccine; Ig, immunoglobulin; Cont., control.

we did not determine the optimum dose for the mice or monkeys, 0.1 μg of PS per mouse and 5 μg of PS per monkey stimulated significantly elevated responses to the PS) at a critical period of perinatal development (e.g., trimester of gestation and/or during lactation), could provide the young with an enhanced immune response to pneumococcal PS immunogens during early life.

Injection of pregnant mice with pneumococcal vaccine induced 6.8- and 2.9-fold IgG and IgM antibody responses, respectively, in young that received an additional dose of vaccine after birth. However, less antibody (2.2- and 1.8-fold increases in IgG and IgM antibodies, respectively) was observed in these infants from mothers given pneumococcal antiserum. Offspring of mothers that received saline also produced a low antibody response (1.9- and 1.6-fold increases in IgG and IgM antibodies, respectively). Regarding the comparison of the antibody responses in offspring whose mothers received antisera and saline, these serum samples were analyzed by using the ELISA at different times; thus they should not be compared directly for their antibody levels.

Similar to the results with the monkey model, there was an enhancement of antibody production in mouse offspring from immunized mothers that received additional pneumococcal vaccine and antiserum (3.94 μg of IgM antibody per ml versus 0.69 μg/ml in the control; 0.74 μg of IgG antibody per ml versus 0.28 μg/ml in the control; $P < 0.01$). Pneumococcal PSs have been observed to induce different antibody responses in different species. For example, in rabbits injection with type 19F and 19A PSs produces extensive cross-reactive antibodies (17); whereas in humans group 19 PSs produce only low levels of cross-reactive antibodies. Therefore, the results of antibody response in one species may not be directly applicable to another species.

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